THE MOSAIC STRUCTURE OF RED BLOOD CELL AGGLUTINOGENS

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The existence of certain structures designated as agglutinogens on the red cell envelope has been inferred from the reactions, hemolysis and agglutination, which occur on contact with specific antisera. These structures, whose chemical nature is incompletely understood, are antigenic, that is, they have the capacity to stimulate the formation of immune antibodies, and the reacting substances or antibodies in the antisera are variously designated as agglutinins or hemolysins. It is now known that red cells are characterized by multiple rather than by single agglutinogens, at least one of which is species specific in that all members of a species appear to share it. Other agglutinogens are type specific and are encountered only in a certain percentage of the individuals of a species. It is with these type specific agglutinogens, the blood group antigens, that we are primarily concerned here.

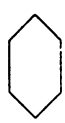
Blood Group Agglutinogens and Antibodies

Blood group antigens have been demonstrated in almost all species examined to date (43). In some cases, the presence of the agglutinogens has been demonstrated with the aid of antibodies occurring naturally in the sera of other animals either of the same species (isoantibodies) or of another species (heteroantibodies). In most cases the reagents have been obtained by deliberate or accidental isoimmunization or by heteroimmunization. In human beings, the A-B-O blood factors are examples of agglutinogens that are detected with the use of naturally occurring isoimmune bodies; the Rh-Hr blood factors, on the other hand, are generally demonstrated with antibodies produced by isoimmunization; while the M-N blood factors are examples of agglutinogens that are tested for with heteroimmune sera. When the blood factors in any one species are studied, they are found to fall into systems like the A-B-O, M-N-S, and Rh-Hr systems in man. Furthermore, the agglutinogens of a system are transmitted by a series of corresponding allelic genes, and, as will be shown later, this phenomenon is exhibited also by lower animal species. In considering the reactions of each agglutinogen with its antisera, the simplest interpretation postulates a one-to-one correspondence between agglutinogens and antibodies. This view, which was held by the earlier workers in the field, is still widely adhered to today but does not conform with available serological facts and must be revised.

Nature of Serological Specificity

Certain principles enunciated by Landsteiner (12) are pertinent for this discussion. To elucidate the reactions of red cells and bacterial antigens of un-

known chemical structure, he conducted investigations on artificial antigens prepared from simple substances of known chemical structure. By diazotizing simple chemicals to egg albumin he prepared complex antigens which he utilized for the production of immune sera. These antisera in turn were tested *in vitro* against the diazotized compound, this time conjugated with horse serum globulin in order to be sure that the reactions obtained were directed against the substance he was studying rather than the protein part of the artificial antigen. In this way he was able to show that a single compound can elicit a multiplicity of antibodies. For example, when immune sera were prepared for *meta*-aminobenzene sulfonic acid, cross reactions were obtained with *para*-aminobenzene sulfonic acid with *ortho*-aminobenzene sulfonic acid (15). When the sera were tested with antigens made of compounds whose molecular structure was the same as that of the original antigen except that the acid group was substituted for by an homologous group (AsO_3H_2 or COOH for the SO_3H group, as in aminobenzene arsenic acid and in aminobenzoic acid), cross reactions were obtained when the homologous radical was in the *meta* position but not when it was in the *para* or *ortho* position. By adsorption experiments it was possible to show that the immune sera contained more than one antibody.

In other experiments it was possible to correlate immunological behavior with stereo configuration, and this in turn could be correlated with crystal characteristics (1a, 21). For example, the halogens and the CH_3 group are mutually replaceable in compounds capable of mixed crystal formation, and the two compounds benzene  and thiophene  can also form mixed crystals. Substances which form mixed crystals will, as a rule, give cross reactions in serological tests, and Landsteiner and van der Scheer (14) indeed demonstrated an immunological relationship among benzene, thiophene, and furane. These and many similar experiments show that a single antigen can elicit the production of multiple antibodies, and, on the other hand, a single antibody can cross react with several antigens of related structure. Thus, the idea of a one-to-one correspondence between antigen and antibody is an idealized concept, which is experimentally refuted even by the simplest of antigen-antibody reactions.

Serological Factors

From the preceding discussion, it is evident that serological reactions give little insight into the chemical or physical structure of an antigen. A single antigen molecule can react with several antibodies of different specificities, and the unknown attributes of the antigen molecule which determine these serological reactions may be termed "serological factors" of the antigen. Since even a single simple antigen may have many such "serological factors", antigens behave in general as if they have mosaic structures. This statement can be readily shown to apply also to antigens whose structure is completely unknown, notably the blood group antigens.

Even less is known regarding the structure of antibodies which accounts for their specific serologic behavior. Antibodies in general are modified serum globulins, but the exact nature of the modification is not known since normal serum globulins and immune serum globulins cannot be distinguished chemically and behave identically when used as antigens. According to one view (1, 29a) antibodies are formed by a steric modification of the surface of the globulin molecule to conform with the surface of the antigen, so that the polar groups on the antibody correspond with the oppositely charged polar groups on the antigen. According to Campbell (5) the active patch responsible for the specificity of an antibody occupies only about one per cent of the surface of the coiled antibody globulin molecule.

Mosaic Structure of the Agglutinogens A and B

Although it was shown a long time ago (43) that the agglutinogens A and B behave serologically as though they have a mosaic structure, this fact seems to have been overlooked in recent years. For example, qualitative differences exist among the anti-B agglutinins in human sera. All anti-B sera give parallel reactions in tests with human blood, that is, they all clump blood containing the agglutinogen B and fail to clump blood that lacks this agglutinogen. However, when the cross reactions of different anti-B sera with animal bloods were studied, interesting differences were found. An agglutinogen related to the agglutinogen B is present in rabbit blood. By absorption tests it was found that while some human anti-B sera could be adsorbed by small amounts of rabbit red cells as well as human red cells, other anti-B sera could not be completely adsorbed even by large amounts of rabbit blood (11). These observations are best explained by postulating the existence of at least two anti-B agglutinins, namely, anti-B_i which reacts with human B blood but not rabbit cells, and anti-B_{ii} which reacts about equally with rabbit red cells and with human B cells. As is to be expected immune rabbit sera for human B blood contain anti-B_i alone; on the other hand, chicken immune sera for human B blood appear to contain only anti-B_{ii}. By further studies on anti-B sera with the blood of other animals such as guinea pigs, further varieties of anti-B agglutinin, anti-B_{iii}. . . . , were demonstrated. Since all of the beta antibodies react with every human B blood, it is evident that the human agglutinogen B behaves as if it has a mosaic composition $B_iB_{ii}B_{iii}$. . . .

Similarly, agglutinogen A exhibits a complex serological behavior. Certain immune rabbit sera for human A blood hemolyze sheep blood, and, on the other hand, certain sheep antisera react strongly with human A cells (29). Thus, agglutinogen A has a Forssman component which has been designated F_A. The situation is further complicated when one studies the subgroups of A. The two main variants of A agglutinogen, A_1 and A_2, behave serologically as if they have a major component in common. In addition, the former has a special blood factor A_1 absent from the latter. Blood of subgroup A_2, on the other hand, has a special factor which also occurs in group O blood. Finally agglutinogens A and B share a component, designated C, which is absent from

group O blood. Accordingly, our present limited knowledge concerning the A-B-O agglutinogens may be summarized as shown in table 1.

Genetically each agglutinogen behaves as a unit and is inherited *in toto*. Its various serological properties, namely, the blood factors, do not segregate, and as has been indicated, do not necessarily represent distinct substances or chemical structures within the agglutinogen molecule. They may, for example, represent a certain pattern of electric charges shared in common with many other agglutinogens (fig. 1). Hence, it is important to distinguish between the use

TABLE 1

Serological properties of the A-B-O agglutinogens

AGGLUTINOGEN	PARTIAL ANTIGENS OR BLOOD FACTORS
B	B_i, B_{ii}, B_{iii}, ... C
A_1	A, A_1, F_A, C
A_2	A, O, F_A, C
O	O

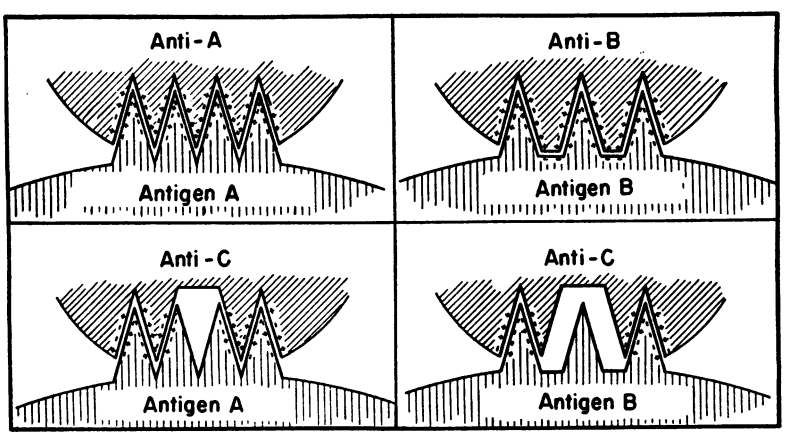


FIG. 1. DIAGRAMMATIC REPRESENTATION OF THE NATURE OF CROSS-REACTIONS
Anti-C combines with two different antigens, A and B, even though neither A nor B contains a definite structure C. Factor C appears to be merely an arrangement of charges on the surface of the antigen molecules shared by A and B.

of the terms blood factors and agglutinogens. The agglutinogen is a definite hereditable substance on the surface of the red blood cell, while the blood factors are only some of the attributes of the agglutinogens that may be demonstrated with appropriate antisera.

In the case of the A-B-O groups, it has been necessary to postulate a series of at least four allelic genes corresponding to the four major agglutinogens (2, 34). The convention among American geneticists is to designate allelic genes at the same locus by the same letter with qualifying superscripts. For example, it has been suggested by Strandskov (33) that the blood group genes be called

I^{A_1}, I^{A_2}, I^B, and I^O, respectively, the letter I being derived from the word "isoagglutinogen". However, in the older literature, the symbols A_1, A_2, B, and O have been used for the genes, with italics to distinguish the genes from the agglutinogens. Most workers prefer these simpler designations even though they do not conform with genetic convention. Actually, when testing the blood of an individual, one is determining the combined effects of a pair of genes (cf. table 2), so that the actions of the individual genes can be deduced only by comparing the reactions of bloods from individuals whose genotypes are known from family studies.

To determine whether two different blood factors are parts of the same agglutinogen or belong to different agglutinogens, family studies must be done. With the blood group AB the factors A and B segregate, only one or the other being transmitted to each child so that these obviously belong to separate agglutinogens. On the other hand, the partial antigens A, A_1, F_A, etc. of ag-

TABLE 2

The six blood groups and ten corresponding genotypes

BLOOD GROUP (PHENOTYPE)	REACTIONS WITH ANTISERA*			CORRESPONDING GENOTYPES
	Anti-A	Anti-B	Anti-A_1	
O	−	−	−	*OO*
A_1	+	−	+	A_1A_1, A_1A_2, and A_1O
A_2	+	−	−	A_2A_2 and A_2O
B	−	+	−	*BB* and *BO*
A_1B	+	+	+	A_1B
A_2B	+	+	−	A_2B

* Anti-O serum is not included in this table because it is not available for routine testing.

glutinogen A_1 are inherited *en masse*, so that these blood factors are parts of a single unit agglutinogen (53a).

Nomenclature. In designating the four blood groups, before the subgroups of A had been found, Moss and Jansky suggested numbering them as I, II, III, and IV, but their two methods of numbering were different, I and IV being interchanged. The use of numerals had an immediate appeal and was popular because of its simplicity. No knowledge or understanding of the fundamentals of the subject was required or expected of individuals using these designations. However, the interchange of numbering led to several transfusion accidents, and workers had no way of knowing about the agglutinogens and agglutinins which characterized each group, except through sheer memory. Moreover, the designations could not be logically extended to include the subgroups of A. It was inevitable, therefore, that the International Nomenclature, which is a modification of the original Landsteiner nomenclature, should finally replace the Moss and Jansky numberings. Still, nothing short of the exigencies of World War II succeeded in bringing this about.

The purpose of a name is to identify and not necessarily to describe fully,

since it is quite impossible to include in a name everything that is known about a subject. If one of the criteria for a happy choice of name were that it include details, it would be necessary to change the name every time information expanded. For example, the symbol A_1 is sufficient to identify the agglutinogen which contains the blood factors A, A_1, F_A, and C, and similarly it is not necessary to include in the designation of the agglutinogen B all of its serological properties. Some workers have suggested that the designations of the four blood groups include the agglutinins that are normally found to be present in the various groups as Aβ, Bα, ABo and O$\alpha\beta$. Such designations, aside from being redundant, would sometimes be incorrect, as with infants who often lack the expected isoagglutinins.

Mosaic Structure of the Agglutinogens M and N

In the case of the M-N types, the original studies (13) appear to indicate a simple structure of the agglutinogens and a simple heredity by a corresponding pair of allelic genes M and N, or as Strandskov has suggested L^M and L^N. Thus, there are three genotypes corresponding to the three phenotypes. However, when the agglutinogens M and N in primates were studied by Wiener (42), it was soon found that antisera which gave parallel reactions in tests against human bloods behaved differently against bloods of monkeys and apes, even though their titers were similar in tests on human bloods. It was found that some anti-M sera agglutinated blood from apes as well as many species of monkeys, while other anti-M sera agglutinated ape blood but not monkey blood. Still others agglutinated human blood of types M and MN, but not blood from apes or monkeys. By comparing the reactions of a number of anti-M sera when tested against blood from lower primates, as many as five different anti-M agglutinins were demonstrated (16). The corresponding blood factors were all present in the agglutinogen M of human blood, which therefore had the mosaic structure $M_iM_{ii}M_{iii}M_{iv}M_v$. . . . Similarly, at least two components of agglutinogen N were shown to exist in human blood, so that this agglutinogen has the structure N_iN_{ii}. . . . In heredity studies it was found that the blood factors $M_iM_{ii}M_{iii}$-$M_{iv}M_v$. . . . do not segregate; the blood of individuals of types M and MN contains all of the M components; and, similarly, blood of types N and MN contains all the components of agglutinogen N.

A further complication arose when Walsh and Montgomery (38) discovered a hitherto undescribed agglutinin that gave reactions which did not correspond with any known A-B-O, Rh-Hr, M-N, or P antibody. Sanger and Race (28) observed, however, that the agglutinin, described as anti-S by them, gave a different percentage of positive reactions depending upon the M-N types of the bloods tested. While the frequency of positive reactions was 73.4 per cent among type M individuals, 54.1 per cent positive reactions were obtained in type MN, and only 32.3 per cent in type N. Thus, the blood factor S was evidently related to the agglutinogens M and N. In family studies (28) it was found that the blood factor S was inherited as a simple Mendelian dominant and appeared to be linked to the genes M and N. For example, if an individual

had inherited the blood factors M and S from the father and N and s from the mother, all children to whom that individual transmitted the blood factor M acquired the factor S at the same time, while those who inherited the blood factor N did not. Sanger and Race considered that the M-N-S types were inherited by pairs of tightly linked gene couplets, and though they also mentioned the possibility of multiple alleles, did not favor the latter alternative.

TABLE 3

The M-N-S genes and agglutinogens

GENETIC SYMBOLS		SYMBOLS FOR AGGLUTINOGENS		BLOOD FACTORS IN AGGLUTINOGENS
Multiple alleles	Gene couplets	Shorthand symbols	Longhand symbols	
L	MS	L	M.S	M_i, M_{ii}, M_{iii}, ... S
l^S	NS	S	N.S	N_i, N_{ii}, . . . S
l^M	Ms	M	M.s	M_i, M_{ii}, M iii, . . s
l	Ns	l	N.s	N_i, N_{ii}, ... s

TABLE 4

The nine M-N-S types and their ten corresponding genotypes

3 M-N TYPES			9 M-N-S TYPES				GENOTYPES	
Designation	Reaction with sera		Designations		Reaction with sera		Longhand symbols	Shorthand symbols
	Anti-M	Anti-N	Longhand	Shorthand	Anti-S	Anti-s		
M	+	−	M.S	LL	+	−	*MS/MS*	*LL*
			M.Ss	LM	+	+	*MS/Ms*	Ll^M
			M.s	MM	−	+	*Ms/Ms*	l^Ml^M
MN	+	+	MN.S	LS	+	−	*MS/NS*	Ll^S
			MN.Ss	Ll	+	+	*MS/Ns* or *Ms/NS*	*Ll* or l^Ml^S
			MN.s	Ml	−	+	*Ms/Ns*	l^Ml
N	−	+	N.S	SS	+	−	*NS/NS*	l^Sl^S
			N.Ss	Sl	+	+	*NS/Ns*	l^Sl
			N.s	ll	−	+	*Ns/Ns*	*ll*

They cite the recent report of Levine *et al.* (19) of the discovery of anti-s, specific for the contrasting blood group factor s, in support of their interpretation.

If the so-called *S-s* genes were linked to the gene pair *M-N*, one would expect crossing over to occur between them. However, no instance of crossing over has been encountered in family studies to date. Moreover, as a result of crossing over, the frequency of blood factor S at equilibrium would become the same in all three M-N types, so that the very phenomenon which led to the uncovering of the relationship between S and the M-N agglutinogens refutes the linkage-crossover concept. The alternative and more reasonable explanation is that

blood factors S-s and M-N are partial antigens within the same agglutinogen molecule and that the resulting agglutinogens are inherited not by linked gene pairs, but by a corresponding series of four allelic genes. In an unpublished paper, Wiener (53) has pointed out how a simple nomenclature incorporating these concepts could be devised, and this is summarized in table 3.

In view of the novelty of the M-N-S types a complete table showing all nine possible phenotypes and the ten corresponding genotypes may be of interest. This is presented in table 4 which at the same time compares the longhand and shorthand methods of designation.

TABLE 5

Classification of the blood factors in the A-B-O, M-N, and Rh-Hr systems

SYSTEM	MORE ANTIGENIC FACTORS	LESS ANTIGENIC FACTORS
A-B-O	A and B	O
M-N-S	M and S	N and s
Rh-Hr	Rh_0, rh′, and rh″	hr′ and hr″

TABLE 6

The eight Rh blood types

Rh_0-NEGATIVE TYPES				Rh_0-POSITIVE TYPES			
Designations (Phenotypes)	Reactions with sera			Designations (Phenotypes)	Reactions with sera		
	Anti-Rh_0	Anti-rh′	Anti-rh″		Anti-Rh_0	Anti-rh′	Anti-rh″
rh	−	−	−	Rh_0	+	−	−
rh′	−	+	−	Rh_1	+	+	−
rh″	−	−	+	Rh_2	+	−	+
rh′rh″ (or rh_y)	−	+	+	Rh_1Rh_2 (or Rh_z)	+	+	+

Mosaic Structure of the Rh-Hr Agglutinogens

The Rh-Hr types constitute the most complex system of human blood factors in man found to date. This complexity has been a source of considerable confusion, though the principles are not different from the A-B-O and M-N-S systems. The chief difference is in the number of blood factors and corresponding antisera involved, as many as six antisera of different specificity being available for the Rh-Hr tests. The blood factors can be classified into two major subdivisions known as the Rh and Hr factors, respectively (49). In general the Rh factors are more antigenic then the Hr factors, while the Hr factors tend to give a greater "gene dose" effect. Thus in homozygous individuals Hr reactions are strikingly stronger than in individuals that are heterozygous for the Hr factor. This subdivision is not peculiar to the Rh-Hr system as is shown in table 5.

The three most important Rh factors are designated Rh_0, rh′, and rh″, respectively. Of these three factors the Rh_0 factor, which was the first found (17),

is the most antigenic and is the most frequent source of clinical complications. The Hr factors are of less clinical importance and are reciprocally related to the Rh factors in the same way that N is related to M, and s is to S. Conceivably, three Hr factors could exist, but to date only two have been convincingly demonstrated; at any rate antisera for the third Hr factor (Hr_0) are not available for use. In tests with the three Rh antisera, eight different phenotypes can be differentiated (44) as shown in table 6. Individuals exist with none, or one, or two, or three of the Rh factors. It will be especially noted that blood containing the two Rh factors, Rh_0 and rh′ together, is designated simply as Rh_1, while blood that contains both Rh_0 and rh″ is designated as Rh_2. The reason for the abbreviated symbol is that in family studies (55) it was found that individuals who possessed Rh_0 and rh′ together generally transmitted this combination *en masse* to their offspring, that is, the factors Rh_0 and rh′ in these families do not segregate. Thus, the factors Rh_0 and rh′ in these individuals appear to belong not to separate agglutinogens, but instead behave like partial antigens within a single unit agglutinogen, Rh_1, presumably determined by

TABLE 7

The eight "standard" allelic Rh genes and their corresponding agglutinogens

GENES	AGGLUTINOGENS	BLOOD FACTORS PRESENT
r	rh	hr′ and hr″
r'	rh′	rh′ and hr″
r''	rh″	rh″ and hr′
r^y	rh_y	rh′ and rh″
R^0	Rh_0	Rh_0, hr′, and hr″
R^1	Rh_1	Rh_0, rh′, and hr″
R^2	Rh_2	Rh_0, rh″, and hr′
R_z	Rh_z	Rh_0, rh′, and rh″

corresponding gene R^1. To account for all the observations made in family studies, however, it is necessary to postulate a series of at least 8 allelic genes, r, r', r'', r^y, R^0, R^1, R^2, and R^z, respectively (48). That in some rare families involving type Rh_1 parents, the factors Rh_0 and rh′ do separate is not because of crossing over between linked genes, but is due to the fact that those individuals belong to genotype R^0r'. In other words, agglutinogen molecules exist which possess Rh_0 along with rh′ but without rh″, while there exist other agglutinogens containing Rh_0 without rh′ or rh″, as well as agglutinogens with rh′ but without Rh_0 or rh″. Furthermore, since hr′ is reciprocally related to factor rh′, it is apparent that any agglutinogen which lacks the factor rh′ has in its place the factor hr′, and *vice versa*.[1] The same statement applies to the factor pair rh″-hr″. Thus, the blood factors present in the 8 agglutinogen units determined by each of the 8 standard allelic genes are summarized in table 7.

The complexity of the Rh-Hr factors can be seen to be multiplied when one considers the reactions of heteroimmune sera. In the experiments which led to the discovery of the

[1] The existence of rare exceptions to this rule has recently been reported by Race *et al.* (26).

Rh factor by Landsteiner and Wiener, blood of rhesus monkeys injected into rabbits and guinea pigs evoked the production of an antibody that gave positive reactions with rhesus blood and with human Rh-positive blood (17, 18). On the other hand, anti-Rh sera of human origin gave negative reactions when tested against rhesus blood. Similarly, in tests done on chimpanzees all were found to be Rh negative with anti-Rh sera of human origin, but were positive when tested with certain anti-hr′ sera of human origin (56). The subtlety of the variations involved could hardly be ascribed to a multiplicity of agglutinogens, and is best considered as variations in the complex structure of a single agglutinogen molecule (46). See fig. 2.

As Fisk and Foord (8a) have shown, the absorbed animal anti-rhesus sera strongly agglutinate Rh-negative as well as Rh-positive blood of newborn infants and fetuses, but react with only Rh-positive blood of older children and adults. This observation is most readily explained in line with the concepts presented in this review, and might be conceived of as an example of biochemical ontogeny recapitulating biochemical phylogeny.

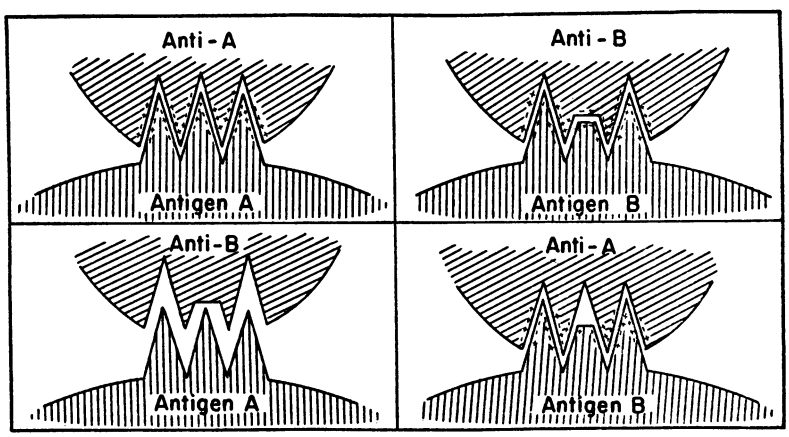


FIG. 2. DIAGRAMMATIC REPRESENTATION OF THE NATURE OF NONRECIPROCAL REACTIONS
Anti-A combines with antigen B almost as well as with antigen A; yet, anti-B combines readily only with antigen B and not with antigen A.

From the outset, even when only the two Rh factors, Rh_0 and rh′, were known, the question arose whether the different Rh factors are inherited by separate pairs of genes, either in different chromosomes or linked within the same chromosome, or whether they are transmitted by multiple allelic genes. Since the same problem had previously arisen and been solved with regard to the A-B-O groups, it was a simple matter to apply the same statistical tests to the Rh types. If Rh_0 and rh′ were inherited by separate genes, either in different chromosomes or within the same chromosome, then at genetic equilibrium the percentage of individuals possessing the rh′ factor should be the same among Rh_0-positive individuals as among Rh_0-negative individuals. Accordingly, the fact that rh′ occurred as a rule in association with Rh_0 and only rarely without Rh_0 makes it most unlikely that there exist separate pairs of genes for these two factors. Therefore, as early as 1942 the concept of separate gene pairs was discarded, and the theory of multiple allelic genes was adopted to account for the inheritance of the Rh blood types (43).

In 1944, however, the theory of separate gene pairs was revived by Fisher and Race (23) who extended it to include the rh″ factor. (At the same time a different method of designating the Rh-Hr factors was suggested, and readers who are interested in a discussion of the nomenclature question may consult previous publications (39, 40, 47, 49, 50, 52) of the present authors.) According to Fisher and Race, the properties Rh_0, rh′, rh″, and hr′ were conceived of not as blood factors, but as complete agglutinogens each determined by a corresponding gene. They postulated the existence of a pair of Rh-Hr chromosomes, each bearing three Rh-Hr genes, namely, one each of the gene pairs, rh′-hr′, rh″-hr″, and Rh_0-Hr_0, which determine the inheritance of corresponding complexes each containing three agglutinogens. A glaring defect of this hypothesis is that no antiserum is available for the demonstration of the postulated agglutinogen Hr_0 of the complex, supposedly present in four of the eight standard possibilities of table 7. Moreover, recent observations which reveal additional varieties of the Rh-Hr agglutinogens further upset this oversimplified concept that the Rh-Hr reactions are inherited in threes. It has been demonstrated that the Rh-Hr agglutinogens do not uniformly contain three blood factors, but may contain as few as one or as many as four.

Callender and Race (4) discovered a fourth antibody for a corresponding Rh factor designated as rh^w. This factor occurs invariably in association with rh′, and almost every example has occurred in an individual of type Rh_1 or Rh_1Rh_2 making it necessary to postulate an additional gene R^{1w} with a corresponding agglutinogen Rh_1^w. This new gene (or chromosome, according to Fisher and Race) determines a complex of not three blood factors but of four, namely, Rh_0, rh′, hr″, and rh^w. More recently still Race *et al.* (26) have demonstrated the existence of a gene[2] which determines a "complex" with only a single blood factor, namely, Rh_0 alone, without rh′, rh″, hr′, or hr″. To keep within the rigid confines of the three gene hypothesis, it has been necessary for the British investigators to postulate that a deletion of the Rh chromosome had occurred involving the gene pairs rh′-hr′, and rh″-hr″. Actually, the fact that the number of blood factors on the Rh-Hr agglutinogen is not uniformly three is readily understood merely as a variation in the mosaic structure of the Rh-Hr agglutinogen, just as variations have been shown to occur in the M-N-S and A-B-O agglutinogens.

With regard to the suggestion that the unusual blood type Rh_0^x resulted from a deletion of the Rh-Hr "chromosome", as indicated by Race's designation —D—, this conflicts with the observation that with only rare exceptions there must be at least one representative of each locus present for development to proceed normally. In *Drosophila* it has been found that large deletions are lethal, while small deletions are viable but only in the heterozygous form when

[2] The gene in question may be designated R^{0z} and the corresponding agglutinogen Rh_0^x. Race *et al.* have used the designation —D— for both the gene and the agglutinogen. Recently, a similar agglutinogen Rh_0^u and corresponding gene R^{0u} has been found in negroids (Wiener and Gordon, unpublished); this would be designated cD— by the Fisher-Race school.

they produce marked phenotypic modifications. (Cf. Sturtevant, A. H., and Beadle, G. W. 1939 An Introduction to Genetics, W. B. Saunders, Philadelphia, Pa.) It is significant that in the case reported by Race *et al.*, the individual was homozygous for the supposed deletion, yet she "enjoys good health".

Chemical Studies; Mode of Action of Blood Group Genes

Little is known concerning the chemical nature of the A-B-O agglutinogen and even less about the M-N and Rh-Hr agglutinogens. The fact that the A-B-O substances occur in water-soluble form in the saliva and other secretions of 80 per cent of individuals (secretors) has made it possible to conduct chemical studies and identify the substances as mucopolysaccharides (43). The inability to extract the M-N and Rh-Hr substances from the red cells, the only place where they are known to occur, has prevented chemical studies. The recent observations that the M-N-S substances appear to be destroyed when red cells are treated with proteolytic enzymes (22, 27, 37) while the Rh-Hr and A-B-O substances are not, suggest that the former owe their specificity to protein-containing material while the A-B-O and Rh-Hr substances do not.

Studies on blood group substances in saliva and gastric juice from normal human beings show the presence of approximately equal amounts of mucopolysaccharides in all individuals. Moreover, the mucopolysaccharides from individuals of different blood groups, or from secretors or nonsecretors, cannot be differentiated by any known chemical test. This suggests that the blood group genes act by bringing about some minor modification in the molecule of a certain substrate which all human beings share in common, namely, the mucopolysaccharides just mentioned. The part of the molecule responsible for the blood group specificity must be relatively minute, just as the modification of the serum gamma globulin responsible for the specificity of an antibody involves only a small portion of the surface of the coiled up antibody molecule. Similarly, the M-N-S and Rh-Hr substances apparently represent minor modifications of substrates common to all human beings, caused by the respective M-N and Rh-Hr genes. The exact function of the A-B-O, M-N, and Rh-Hr substances, if indeed they each have a specific function, is unknown, and, of course, the serological reactions give no indication what they may be. Presumably, they are integral parts of the red cell envelope.

Variants of the Blood Group Factors

As has already been mentioned, in the earlier work on the blood groups it was necessary to postulate the existence of two blood factors, A and B, which could be sharply differentiated from one another. It was later recognized that the blood group factor A was not identical in all individuals and two major variations of this agglutinogen, designated as A_1 and A_2, were identified. Still later, rarer variants of the blood factor A designated as A_3, A_4, and A_5 were found to exist. This general pattern has been duplicated for every blood group system that has been intensively studied, namely, first a number of sharply differ-

entiated blood factors were found, and later it was seen that each blood factor in turn exhibited minor variations.[3]

Whereas the major blood factors of a system all have their corresponding specific antiserums, this is not true of the variants of a blood factor. For example, so-called agglutinogen N_2 is recognized (6, 10) by the fact that while like typical N agglutinogen it reacts with anti-N serum but not with anti-M, not every anti-N serum is capable of reacting with the N_2 agglutinogen, and when an anti-N serum clumps cells containing N_2 the reactions are generally weaker than those of typical N blood. On the other hand, it has not been possible to prepare a special antiserum which will react with N_2 cells and not with typical N blood.

Similarly, in the case of the variants of the agglutinogen A, the agglutinogens A_2, A_3, A_4, etc. are identified not with the aid of special corresponding antisera, but by the progressively weaker degree of clumping observed in tests with a single variety of antiserum, namely, ordinary anti-A (9, 54). While some workers attribute the weaker reactions to the presence in the blood of smaller amounts of the same factor, A or N as the case may be, comparative titrations with a variety of antisera of the same specificity showed that this was not so. For example, if A_2 blood differed from A_1 blood merely in the quantity of agglutinogen A it contained, then the relative titers given by A_1 and A_2 bloods should be the same with all anti-A sera. Actually, some anti-A sera act on A_1 blood and hardly at all on A_2 blood, while others of about the same titer react almost as strongly on A_2 blood as on A_1. Thus, the difference in behavior of the variants of agglutinogen A (or N) is not due to a difference in the quantity of the blood factor they contain, but to some qualitative difference in the structure of the agglutinogen molecule. Significantly, the variants are inherited as such by corresponding allelic genes.

Variants have also been encountered in the Rh-Hr system of blood factors, where certain bloods give intermediate reactions with anti-Rh or anti-Hr sera (45). These variants, the most important of which are the variants of Rh_0, are hereditable (51). When it was observed that some anti-Rh sera clumped ordinary Rh-positive blood, but not blood with an Rh_0 variant, while other Rh_0 antisera clumped both, some workers explained this by postulating that the latter sera contained two distinct antibodies, one for typical Rh_0 blood and the other for the Rh_0 variants (25). This interpretation was a necessary deduction from their concept of one-to-one correspondence between blood factor and antibody. Actually, their own absorption experiments showed that only a single antibody was present in these sera which could be completely absorbed with blood containing either typical Rh_0 or only the Rh_0 variant. Similarly, the idea that the cross reaction of anti-rh′ serum with Rh_1^w blood and typical Rh_1 blood is due to the presence of two antibodies, anti-rh′ and anti-rh^w, could not be substantiated (24). Again absorption experiments show the presence of only a single antibody absorbable by both Rh_1 and Rh_1^w blood.

[3] Also in animals, such as cattle (30) and dogs (57), blood factor variants have been described.

Of interest is the so-called gene dose effect. Blood that is homozygous for a given blood factor generally reacts more intensely with the corresponding antiserum than does heterozygous blood. This is generally interpreted as due to the presence of larger quantities of the blood factor in the homozygous blood. If this were the only reason, however, then all specific antisera should give this effect, but as Malone *et al.* (20) have shown for rh″, for example, not all antisera are suitable for "genotyping". Thus the gene dose effect appears to be due to qualitative as well as quantitative differences in the agglutinogen molecule.

Other Blood Group Systems in Man

As a result primarily of recent work by British investigators,[4] the number of blood group systems known to exist in man has been increased to at least eight. It is unnecessary for the purpose of this review to present an analysis of these systems since they introduce no new principles. In some cases due to the low avidity and specificity of the antisera available some of the observations are uncertain. This statement applies, for example, to the so-called Lewis system and the P blood group system. In other cases the reactions can be elicited only by the anti-globulin technique, as in the so-called Duffy system. The terminology in many cases is as yet unsatisfactory, because the custom has been followed of using the name of the individual with whose blood the antigen was detected, namely, Kell, Cellano, Lutheran, etc. However, a satisfactory terminology cannot be developed until better antisera and methods become available, so that the facts concerning the serology and genetics can be established unequivocally.

Blood Groups in Animals

In conclusion, a few remarks about blood groups in animals are appropriate. The discussion will be limited to the most recent and extensive investigations, namely, the work of Stormont and co-workers (30, 32) on blood groups in cattle and that of Briles *et al.* (3) on blood groups in chickens. The findings in cattle in particular go far beyond the observations that have been made on human blood, but the same general principles are still applicable.

In cattle, blood factors are tested for by isoimmune sera prepared by the injection of blood from one animal into another. The tests are generally carried out not by the agglutination technique but by demonstrating isohemolysis (7, 8, 36). Because of the large number of antigenic blood factors in cattle blood, if transfusions are carried out at random the resulting isoimmune serum is often polyvalent for many blood factors. To limit the number of antibodies in the serum, therefore, transfusions are carried out between donors and recipients selected on the basis of their known blood types, or in earlier studies between parents and their offspring or between siblings, and sera containing single antibodies are prepared by suitable absorption experiments. In this way reagents have been obtained for more than 40 different blood factors.

[4] Race, R. R., and Sanger, R. 1950 Blood Groups in Man. Blackwell Scientific Publications, Oxford. 290 pp.

In heredity studies it was found that each of the blood factors behaves as if it were inherited as a simple Mendelian dominant (7, 8). When members of a family were tested for all known blood factors at the same time, however, it was found that these factors are not all inherited independently of one another but tended to go together in blocks of different sizes (30, 32). For example, as shown in table 8 a sire possessing the blood factors B, C_1, G, I, J, O_1, T_2, P_2, and A′ gave to one half of his offspring the complex $BGIO_1T_2A'$ and to the other half the complex O_1Y_2A' while the blood factors C_1 and J each segregated inde-

TABLE 8

*Heredity of blood group factors in cattle**

Illustrative study showing inheritance of the blood factors B, C_1, G, I, J, O_1, T_2 Y_2 and A′ of sire H1 (Holstein-Friesian) by 20 of his offspring.

MATING NUMBER	DAM'S NUMBER	FACTORS OF THE SIRE POSSESSED BY THE DAMS	PROGENY'S NUMBER	FACTORS OF THE SIRE IN BLOODS OF PROGENY
1	190	— — — — — — C_1 —	231H	B G I O_1 T_2 A′ — — — — J
2	A162	— — — — — — — — —	A255	B G I O_1 T_2 A′ — — — C_1 J
3	C	— — O_1 — A′ — — J	C1	B G I O_1 T_2 A′ — — — C_1 —
4	Z28	— — O_1 T_1 — — C_1 J	Z28C	B G I O_1 T_2 A′ — — — C_1 —
5	80	— — — — — Y_1 C_1 —	80C	B G I O_1 T_2 A′ — — — C_1 —
6	A128	— — — — — Y_1 — J	A284	B G I O_1 T_2 A′ — — — C_1 J
7	10B	— — — — — Y_1 — J	217	B G I O_1 T_2 A′ — — — — J
8	261	B — — — A′ — C_1 —	70H	B G I O_1 T_2 A′ — — — C_1 —
9	8B	— G — — — Y_2 C_1 —	215	B G I O_1 T_2 A′ — — — C_1 —
10	M235	— G — — — Y_2 — —	M282	B G I O_1 T_2 A′ — — — C_1 J
11	A162	— — — — — — — — —	A278	— — — — — — O_1 Y_2 A′ — —
12	127	— — — — — — — — J	9H	— — — — — — O_1 Y_2 A′ — —
13	M185	— — — — — — — — J	M281	— — — — — — O_1 Y_2 A′ C_1 —
14	239	— — — — — — — C_1 J	40H	— — — — — — O_1 Y_2 A′ C_1 J
15	A199	B — O_1 — — — — —	A253	— — — — — — O_1 Y_2 A′ — J
16	256	B — — — A′ — — —	20H	— — — — — — O_1 Y_2 A′ C_1 —
17	357	— — — — — — C_1 —	187H	— — — — — — O_1 Y_2 A′ C_1 —
18	1B	— — — — — — — — —	227	— — — — — — O_1 Y_2 A′ — —
19	49	B — — — A′ — C_1 —	49C	— — — — — — O_1 Y_2 A′ C_1 J
20	Z15	— — O_1 — A′ — C_1 J	Z15C	— — — — — — O_1 Y_2 A′ C_1 —

* After Stormont, Owen, and Irwin (32).

pendently of the other blood factors and of one another. Of the 38 different blood factors studied by Stormont *et al.*, 21 were shown to be members of a system designated as the B system, 7 were members of a second system designated as the C system, while the remaining 10 blood factors appeared to be parts of agglutinogens belonging to other independent blood group systems. In the B and C systems the blood factors were inherited in blocks of divergent sizes forming unit agglutinogens with complex mosaic structures, as in the example shown in table 8. A minimum of 80 allelic genes had to be postulated (31) for the agglutinogens of the B system, and 22 had to be postulated for the genes of the C system, so that the situation is quite comparable to, although

far more complicated than that of the Rh-Hr system in man. It can readily be appreciated that the theory of tightly linked genes as well as the idea of one-to-one correspondence between gene and blood factor and antibody cannot be applied to the blood factors in cattle.

The remarks previously made about nomenclature of blood group genes and agglutinogens are especially pertinent in connection with the work on blood groups in cattle. At first, Stormont tried to include all the blood factors present in an agglutinogen in its symbol, as follows: B^{BO_1}, $B^{BGKO_2Y_1A'E'_3K'}$, $B^{O_3D'E'_3}$, $B^{BGO_1Y_1}$, etc. He soon found that such symbols were too cumbersome to work with, so that shorthand symbols were coined by him for the B and C systems of blood groups in cattle as follows: B, B1, B2, B3, . . . B89; and c, C1, C2, C3, . . . C23, and the reactions of each of these agglutinogens were tabulated and filed with the Pure Breed Dairy Cattle Association. This change was fortunate because more recent studies have disclosed the existence of additional blood factors, so that, for example, the B28 agglutinogen, which reacts with antibodies for factors B, G, K, O_2, O_3, Y_1, Y_2, A′, E'_3, and K′, has been found to possess also factors NF_3 (NF = new factor), NF_4, NF_6, and NF_8. Had the original cumbersome terminology been retained, it would have become necessary to modify the terminology and make it increasingly complex each time a new blood factor was found.

When discussing the blood group substances in cattle, the term "agglutinogen" may seem inappropriate since hemolysis and not agglutination occurs in the *in vitro* tests. This objection can be avoided by adopting the term "hemogen," first suggested by Greval (11a) as an abbreviation of "hemagglutinogen" to describe the group substances both in animals and man.

Similar observations were made by Briles *et al.* (3) in chickens, but here the number of blood factors identified was smaller and the agglutinogen mosaics were correspondingly less complex. As had previously been shown by Wiener (41) from an analysis of the data of Todd (35), the agglutinogens in chickens, as in man and cattle, are inherited by multiple allelic genes.

In summary, it may be stated that the data reviewed appear to be best interpreted as indicating that the blood group agglutinogens both in animals and man are integral parts of the red cell envelope having a complex mosaic structure, the units of which are the various blood factors. The agglutinogens fall naturally into systems, and the genetic mechanism which governs the inheritance of the agglutinogens of each system is one of multiple allelic genes. That the nature of the blood group reactions had been clearly explained by Landsteiner, long before the evidence presented here became available, is another indication of his genius which made him the father of the fields of blood grouping and immunochemistry.

Addendum: While this review was in press, an important paper by B. E. Dodd, "Linked anti-A and anti-B antibodies from group O sera" appeared (Brit. J. Exp. Path., **33**; 1–18, Feb. 1952). The observations of this worker clearly demonstrate the presence in most group O sera of anti-C agglutinins reacting with a corresponding blood factor C in cells of groups A, B, and AB. However, Dodd is apparently unaware of the distinction between blood factors and agglutinogens and adopts instead the old erroneous view of a one-to-one correspondence between agglutinogens and antibodies. Thus, she rejects the more reasonable interpretation, and proposes in place of it a far more involved hypothesis entailing antibody molecules containing both anti-A and anti-B combining groups, the details of which can be found in the original article.

Also, while this review was in press a statement appeared in the *Journal of the American Medical Association* (pp. 211 and 212, May 10, 1952) which summarizes the present status of the Rh-Hr nomenclature question. Interested readers are referred to this comprehensive analysis, which supersedes the one published five years previously in *Science* by the Advisory Board to the National Institute of Health.

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